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Changes in freshwater bacterial community composition during measurements of microbial and community respiration

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The respiration rates of a pelagic community and of its microbial fraction (< 1.2 μm) were measured at two depths in the oxic layer of a meromictic alpine lake (Cadagno, Switzerland) using the oxygen technique. The duration of the incubations were 12, 24 and 55 h. Bacterioplankton abundance (DAPI counts) and composition (whole cell hybridization using 11 group-specific rRNA-targeted oligonucleotide probes) were measured during the incubations. Respiration generally increased with time, especially in the microbial fraction, or remained similar. This result was not always consistent with changes in bacterial abundance and cell volume. The composition of the community also changed during the incubations. The abundance of β-Proteobacteria increased during the course of all the experiments. These results extend the previous conclusions drawn in marine environments to fresh waters and demonstrate that, in addition to changes in bacterial abundance, cell volume and biomass, changes in the taxonomic composition of the bacterial community can occur during discrete incubations of freshwater planktonic communities.

INTRODUCTION

Most biological processes involved in the carbon flux of pelagic aquatic ecosystems are estimated essentially using discrete incubations of communities and by measuring the uptake of a specific compound or changes in the concentration of an element. Even the approaches that are not directly based on incubations, such as remote sensing (Platt *et al.*, 2000) and *in vivo* fluorescence (Falkowski and Kolber, 1993), rely at least in part on empirical relationships derived using discrete incubations.

A prominent example of the use of discrete incubation is the study of the balance between gross primary production (P_g) and respiration (R), which is a major factor determining the role of aquatic communities in the global carbon cycle. Despite their importance in the understanding of ecosystem metabolism (e.g. Biddanda *et al.*, 2001), respiratory processes have received considerably less attention than photosynthetic carbon fixation, which makes it difficult to determine whether a particular system is net autotrophic ($P_g > R$) or net heterotrophic ($P_g < R$) with respect to carbon. For example, a recent review of coastal metabolism revealed only 10 data sets that comprised measurements of P_g and R for both the water column and sediment (Gattuso *et al.*, 1998). The low number of respiration data compared to primary production data is essentially due to technical constraints and limitations. The measurement of primary production by radioisotopic techniques is straightforward [but interpretation of data remains problematic; see (Peterson, 1980)] compared with that of respiration.

The respiration rate is most often estimated by measuring changes in dissolved oxygen (O_2) during the course of *in situ* or laboratory incubations. The rate of respiration of oligotrophic areas or of the microbial fraction of the community is often very low, which makes changes in O_2 difficult to estimate during short-term (a few hours) incubations. Therefore, the samples are commonly incubated for 24 h or more. Times of this duration are of the same order of magnitude as the generation time of microbes. Additionally, it was shown more than 60 years ago that containment provides a large surface area which can be colonized by bacteria (Zobell *et al.*, 1936). Thus, it is not surprising that changes in bacterial abundance can occur during measurements of respiration rates [e.g. (Pomeroy *et al.*, 1994)].

It is critically important to determine whether community composition is also altered during incubations because different bacterial phylogenetic groups exhibit distinct metabolic capabilities, for example utilization of low molecular weight dissolved organic matter (Ouverney and Fuhrman, 1999; Cottrell and Kirchman, 2000). It has recently been demonstrated that bacterial communities change dramatically during long-term (8–10 days) incubations (Massana *et al.*, 2001). These authors further showed that long-term bottle incubations essentially measure the activity of a few opportunistic species rather than that of the original community. Sherr *et al.* (1999) have shown that average metabolic activity of oceanic bacteria in bottle incubations can vary widely compared to the average metabolic activity of *in situ* bacterioplankton. The aim of this paper is to investigate simultaneously the changes in bacterial community composition as well as community and microbial respiration during incubations of biogeochemically-relevant durations.

METHOD

Experiments were carried out in Lake Cadagno, a meromictic lake located in southern Switzerland (Del Don *et al.*, 2001). Ten litres of lake water were sampled in September 1999 in the top (1 m) and the bottom (6 m) of the oxic layer using a 'Friedinger'-type bottle (Züllig AG, Switzerland). The average temperatures were 14.6°C and 13.7°C, and the concentrations of chlorophyll *a* were 4.9 and about 2 μg l^{-1} (Bossard and Lehman, personal communication) at 1 and 6 m, respectively. At each depth, 5 l were immediately distributed into 60 ml biological oxygen demand (BOD) bottles (overflowing > 60 ml). Four bottles were fixed immediately with Winkler reagents, two bottles were brought back to the laboratory for determination of microbial community composition, and the remaining bottles were incubated in darkness *in situ* at 0.3 m depth (temperature: 14.7°C). The remaining 5 l were brought back to the laboratory and filtered onto 1.2 μm membranes under a low vacuum (1 Pa). A second set of BOD bottles was filled with filtered lake water from each depth, processed and incubated as described above. Two to six BOD bottles were fixed with Winkler reagents 10–14 h, 21–25 h, and 52–57 h after the beginning of the incubation (for convenience, these time ranges will be referred to as 12, 24 and 55 h). Finally, two bottles were used at the end of the incubation for determination of microbial community composition.

The oxygen concentration was titrated at 0, 12, 24 and 55 h using an automated Winkler titration technique with potentiometric end-point detection (Anderson *et al.*, 1992) and an Orion redox electrode (9778-SC). Reagents and standardizations were similar to those described by Knap *et al.* (Knap *et al.*, 1996). The rate of respiration and its standard error were determined by regressing O_2 against time during the following time intervals: 0–12 h, 12–24 h, 24–55 h and 0–55 h.

The bacterial community composition was investigated as described by Tonolla *et al.* (Tonolla *et al.*, 2000) on subsamples (30 ml) taken at times 0 and 55 h. The samples were filtered through 0.22 μm polycarbonate membranes (25 mm diameter; Millipore, Bedford, USA). The filters were transferred into 1.5 ml Eppendorf tubes containing 1 ml of 3% paraformaldehyde in phosphate-buffered saline (PBS; 0.13 M NaCl, 7 mM Na_2HPO_4, 3 mM NaH_2PO_4, pH 7.2). Bacterioplankton was released from the filters and resuspended by agitation using a Vortex mixer for 2 min. at 2000 r.p.m. The total release of bacteria from polycarbonate membranes was checked microscopically after DAPI staining. After fixation at 4°C for 16 h, cells were washed twice in PBS and stored in 50% ethanol in PBS at –20°C. *In situ* hybridization with fluorescent (Cy3-labeled) oligonucleotide probes was performed on aliquots (1 μl) of paraformaldehyde-fixed water samples spotted onto gelatin-coated slides (0.1% gelatin, 0.01% $KCr(SO_4)_2$), hybridized and concomitantly stained with DAPI according to Zarda *et al.* (Zarda *et al.*, 1997). Eleven group-specific ribosomal RNA (rRNA) -targeted oligonucleotidic probes were used (Table I). Probes EUK502 and cEUB were used, respectively, as a control of the efficiency in removing eukaryotes in the filtered samples and as a negative control for non-specific binding. Members of the phylum Cyanobacteria were counted separately based on the bright orange autofluorescence of phycoerythrin following excitation at 552 nm and its characteristic morphology.

Direct counts of bacteria were made using a Zeiss Axiolab (Zeiss, Germany) epifluorescence microscope using filter sets F31 (AHF Analysentechnik, Germany; D360/40, 400DCLP, D460/50) and F41 (AHF Analysentechnik; HQ535/50, Q565LP, HQ610/75). Microorganisms were counted at 1000× magnification in 40 fields covering an area of 0.01 mm^2 each. Numbers are expressed as mean ± standard error (SE) and all probe-specific cell counts are presented as the percentage of cells visualized with DAPI. Bacterial morphotypes were analysed on images captured with a charge-coupled device camera (CF 8/1 FMC, Kappa, Germany) connected to the epifluorescence microscope and using the Q500MC Image Processing and Analysis System (Leica, UK). Thirty to 300 cells were measured. Apparent cell volumes were estimated by calculating the volume of the best-fitting ellipsoid.

All statistical analyses were performed using R for MAC OSX. The effects of depth, filtration and time of

Table I: Probes used targeting 16S rRNA or 23S rRNA sequences

Probe	Specificity	Sequence (5′-3′) of probe	Target site (rRNA positions)	FA	Ref.
EUK502	Eukarya	ACCAGACTTGCCCTCC	16S rRNA (502–516)	20	1
ARCH915	Archaea	GTGCTCCCCCGCCAATTCCT	16S rRNA (915–934)	20	2
EUB338	Bacteria	GCTGCCTCCCGTAGGAGT	16S rRNA (338–355)	30	1
cEUB	Target sequence of probe Eub338	ACTCCTACGGGAGGCAGC		30	3
ALF1b	α-subdivision of proteobacteria	CGTTCGYTCTGAGCCAG	16S rRNA (19–35)	10	4
BET42a	β-subdivision of proteobacteria	GCCTTCCCACTTCGTTT	23S rRNA (1027–1043)	30	4
GAM42a	γ-subdivision of proteobacteria	GCCTTCCCACATCGTTT	23S rRNA (1027–1043)	30	4
SRB385	δ-subdivision of proteobacteria	CGGCGTCGCTGCGTCAGG	16S rRNA (385–402)	20	5
SRB385Db	Desulfobacteriaceae	CGGCGTTGCTGCGTCAGG	16S rRNA (385–402)	30	6
CF319a	*Cytophaga–Flavobacterium*	TGGTCCGTGTCTCAGTAC	16S rRNA (319–336)	35	7
HGC69a	Gram-positives with high GC DNA content	TATAGTTACCACCGCCGT	23S rRNA (1901–1918)	35	13

FA is the formamide concentration (% v:v) in the *in situ* hybridization buffer. The target site is given with reference to *Escherichia coli* numbering. The key to references (Ref.) is as follows: 1, (Amann *et al.*, 1995); 2, (Stahl and Amann, 1991); 3, (Wallner *et al.*, 1993); 4, (Manz *et al.*, 1992); 5, (Amann *et al.*, 1990a); 6, (Rabus *et al.*, 1996); 7, (Manz *et al.*, 1996); 8, (Roller *et al.*, 1994).

incubation were determined using a three-way analysis of variance (ANOVA).

RESULTS

The ranges of the respiration rates of the whole community and filtered fractions were, respectively, 0.07–0.32 and 0–0.15 μmol O_2 l^{-1} h^{-1} (Figure 1). The initial rate of respiration of the whole community was higher at 1 than at 6 m. Respiration exhibited significant changes as a function of incubation time at both depths. It was not detectable for the filtered fraction during the first 24 h, neither at 1 nor 6 m, and became significant during the period 24–55 h (0.15 ± 0.02 and 0.06 ± 0.004 μmol O_2 l^{-1} h^{-1}, respectively at 1 and 6 m). Respiration of the whole community fraction sampled at 1 m remained relatively constant during the first 24 h of incubation (average: 0.25 μmol O_2 l^{-1} h^{-1}) and decreased by 57% (0.12 μmol O_2 l^{-1} h^{-1}) afterwards. The whole community sampled at 6 m exhibited a similar change as a function of incubation time except that its rate of respiration was not significant during the first 12 h. It subsequently peaked between 12 and 24 h (0.32 μmol O_2 l^{-1} h^{-1}) and decreased by 52% (0.21 μmol O_2 l^{-1} h^{-1}) between 24 and 55 h. As a result of these differential changes in the respiration rates of the whole community and the filtered fraction, the contribution of the microbial fraction (< 1.2 μm) to the total respiration exhibited considerable changes during the course of the incubation. It was not measurable at either depth during the first 24 h and increased to 128 and 32%, respectively at 1 and 6 m, during the last period of measurement (24–55 h). The average contribution during the whole incubation time (55 h) was 42% at 1 m and 23% at 6 m.

There was a significant effect of depth ($P = 0.003$) and time of incubation ($P < 0.001$) on the bacterial abundance but the effect of filtration was not significant ($P = 0.84$). Its value was significantly higher at 6 m than at 1 m in the unfiltered samples (Figure 2). The bacterial abundance of the samples collected at the two depths exhibited a similar behaviour during incubation. It increased initially, peaked after 12 to 24 h and decreased afterwards. It increased by 8 to 23% during incubation of the whole community (1.3×10^6 to 1.6×10^6 cells ml^{-1}) and the filtered fraction (1.2×10^6 to 1.3×10^6 cells ml^{-1}) of the samples collected at 1 m. In contrast, the bacterial abundance of samples

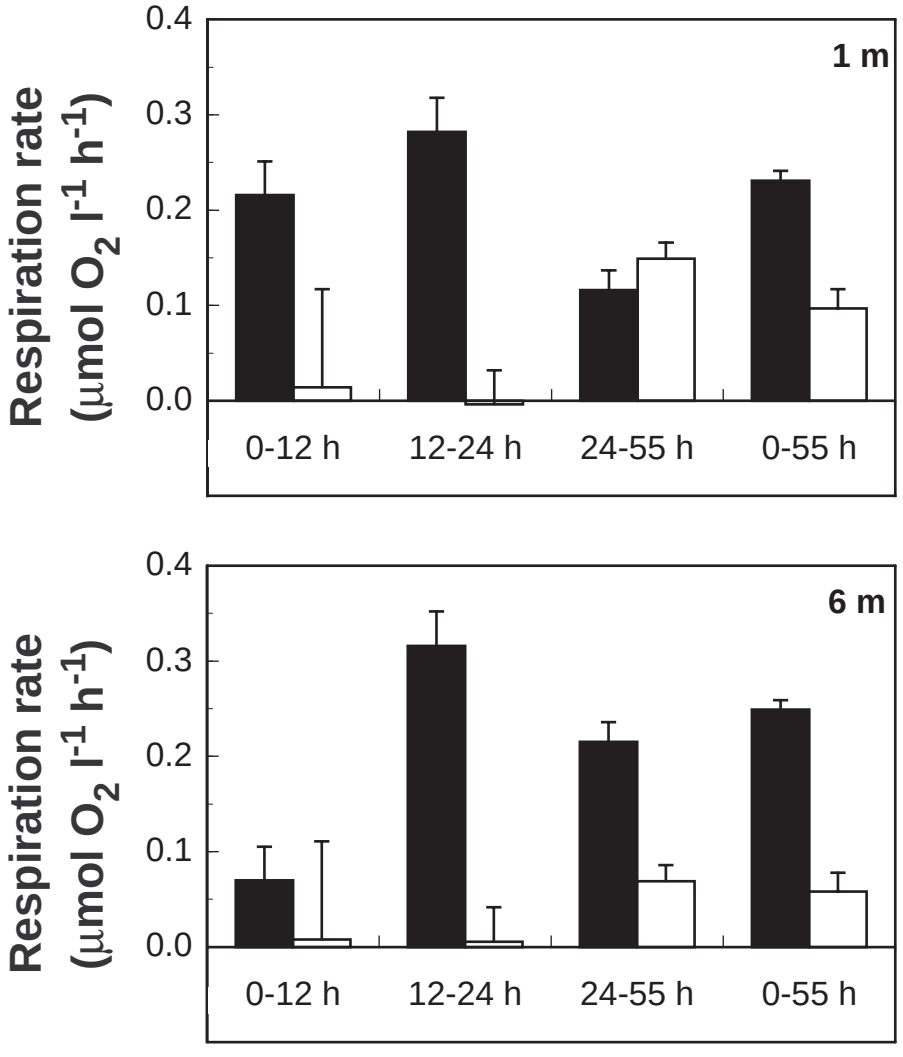

Fig. 1. Respiration rate of the whole community (black columns) and of the fraction filtered on 1.2 μm membranes (white columns) as a function of the incubation period. Values are mean ± SE.

collected at 6 m depth decreased by approximately 30%, both for the whole community (1.6×10^6 to 1.1×10^6 cells ml^{-1}) and the filtered fraction (1.3×10^6 to 0.9×10^6 cells ml^{-1}). The bacterial cell volume was significantly affected by the time of incubation ($P < 0.001$) but not by the sampling depth ($P = 0.51$). It increased significantly during the course of the incubations in all samples (Figure 3; range: 27–118%). This was the result of a decreased abundance of small bacteria (length < 0.6 μm) and an increased abundance of medium-sized morphotypes (length of ca. 0.8–1.2 μm).

Probes EUK502 and cEUB (reverse complement of probe EUB338) were applied as controls for the presence of eukaryotic cells and the absence of false-positive bacterial cells respectively. Probe EUK502 detected only some phytoplanktonic cells that resisted the fixation procedure with the paraformaldehyde buffer and ethanol; it is likely that protozoans were destroyed by the application of that procedure and were therefore not observed. The major bacterial groups contributing to the count of detectable bacteria were the β-subdivision of Proteobacteria (Figure 4; 9.5 ± 5%) and the Cyanobacteria cluster (6.6 ± 2%). The γ-Proteobacteria did not exceed 0.4%, except for the filtered sample from 1 m depth where bacterial cells positive to probe GAM42a increased from 0.3 to 16.7% during incubation. Members of the α-subdivision of Proteobacteria and of the *Cytophaga–Flavobacterium* cluster showed a slight increase in both the unfiltered sample of 6 m depth and the filtered sample of 1 m depth (Figure 4). Members of the δ-Proteobacteria,

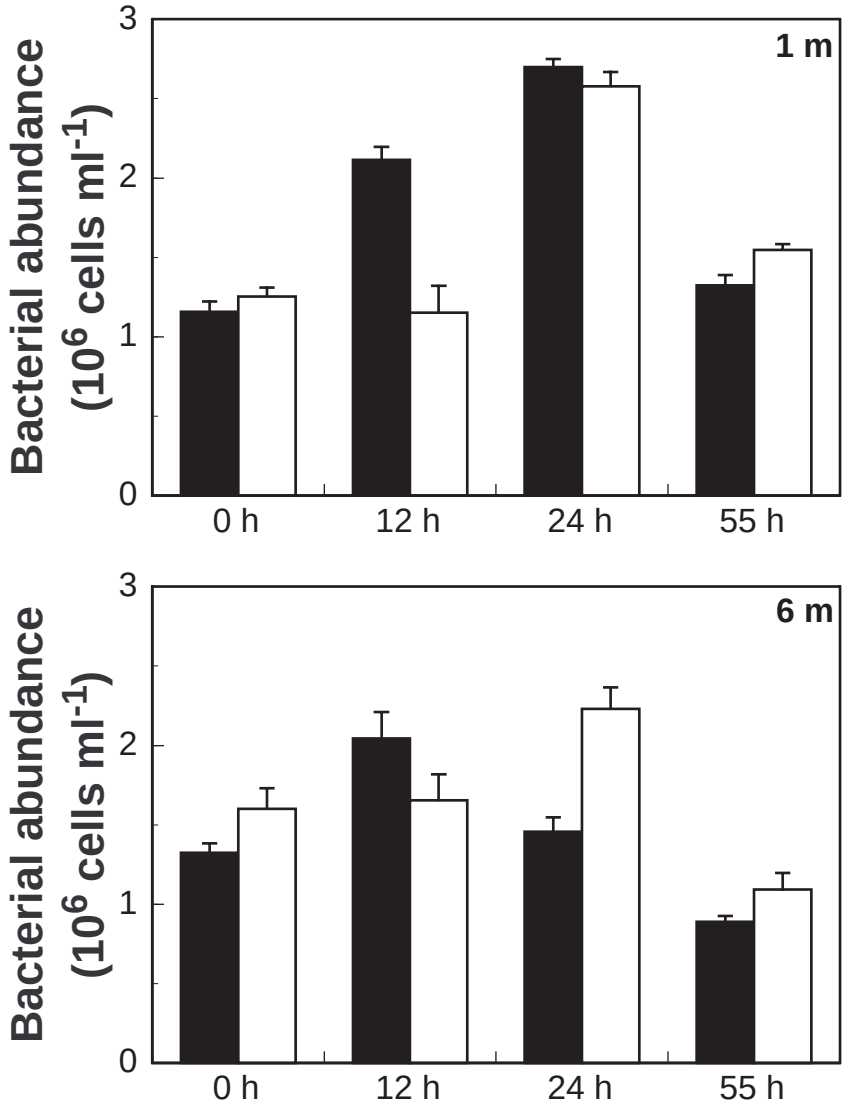

Fig. 2. Changes in the abundance of DAPI-stained bacteria during incubations of the whole community (black columns) and of the fraction filtered on 1.2 μm membranes (white columns). Values are mean ± SE.

of the Gram-positive bacteria with high GC content and of the Archaea showed abundance lower than 0.4% during the whole experiment in all samples. The community composition was relatively similar at 1 and 6 m. The whole community and the filtered fraction exhibited slight differences in community composition. For example, the β-Proteobacteria of the samples collected at 6 m were less abundant in the filtered fraction than in the whole community (2 vs. 11%). The abundance of the *Cytophaga–Flavobacterium* cluster also decreased in the filtered fraction of 1 m depth samples (0.6 vs. 1.5%).

The bacterial community composition changed during incubations. The percentage of cells positive to the Eubacteria probe but unaffiliated with one of the specific groups (referred to as 'Others' in Figure 4), decreased in all samples from 32 ± 3% in the initial samples to 23 ± 4% after 55 h of incubation. The abundance of bacteria belonging to the β-subdivision of Proteobacteria increased in all samples; the increase factor ranged from 1.2 to 3.5. The α-Proteobacteria also increased in another sample (from 1.1 to 2.7%).

γ-Proteobacteria increased dramatically in one sample (filtered fraction from 1 m depth), due to the appearance of a rod-shaped bacterium (dimensions: 1.6 × 0.7 μm). The number of different bacterial morphotypes within the α-, β- and γ-subdivisions of Proteobacteria and the

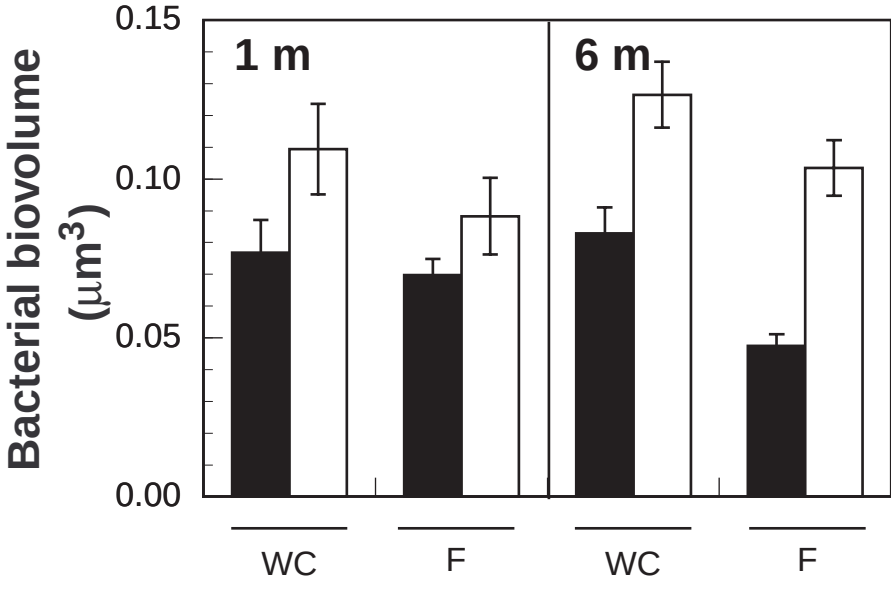

Fig. 3. Cell volume of DAPI-stained bacteria at the beginning of the incubations ($T_{0\,h}$, black columns) and after 55 h ($T_{55\,h}$, white columns). WC, whole community; F, fraction filtered on 1.2 μm membranes. Values are mean ± SE.

Cytophaga–Flavobacterium cluster remained constant during incubation (Table II) but their respective abundance varied. In the initial samples from 1 m depth, six of the 14 morphotypes of β-Proteobacteria dominated the population and were all 1.1 to 2.2 μm in length. A new morphotype (1.5 × 0.7 μm) appeared after 55 h of incubation and became dominant. Moreover, in the unfiltered samples from 6 m depth, three morphotypes were apparently lost during incubations and a new one (1.8 × 0.7 μm) appeared and became dominant. Cyanobacteria were present with two dominant *Synechoccus* spp.-like morphotypes.

Table II: Number of morphotypes identified in the major bacterial group investigated in unfiltered water samples

	1 m		6 m	
Probes	$T_{0\,h}$	$T_{55\,h}$	$T_{0\,h}$	$T_{55\,h}$
ARCH915	6	9	6	6
ALF1b	7	7	7	4
BET42a	13	14	13	11
GAM42a	4	6	3	3
SRB385	6	5	4	5
CF319a	5	4	5	6
Cyanobacteria	3	3	3	3

$T_{0\,h}$, initial population; $T_{55\,h}$, population after 55 h of incubation.

DISCUSSION

The respiration rate, as well as bacterial abundance and cell volume, exhibited significant changes during incubations of filtered and unfiltered samples collected at 1 and 6 m in the meromictic Alpine lake Cadagno. Measurements of pelagic respiration are typically carried out during incubations of 24 h or more (Pomeroy *et al.*, 1994). Respiration can decrease during the course of incubation when resource limitation occurs. Decreases in unfiltered samples can involve a decreased abundance of bacterial cells resulting from a grazing activity higher than bacterial growth rate whereas limitation of unfiltered fractions can result from low concentrations of organic carbon and/or nutrients (Williams, 1981). In contrast, the respiration rate can increase during incubations when neither the bacterial growth rate is resource-limited nor the bacterial concentration is grazing-limited. Changes in the respiration rate during incubation of pelagic communities has been reported previously. In a thorough study carried out in several coastal and off-shore marine sites, Pomeroy *et al.* reported non-linear decreases in dissolved oxygen and frequent exponential increases in bacterial production over time-scales used to measure respiration (Pomeroy *et al.*, 1994). They also found that the bacterial abundance, and often the bacterial size, at the end of incubation were higher than those at the beginning of incubation. The likelihood of changes in respiration as well as number and size of bacteria increases as a function of increasing incubation time. Carignan *et al.* found a decrease in the

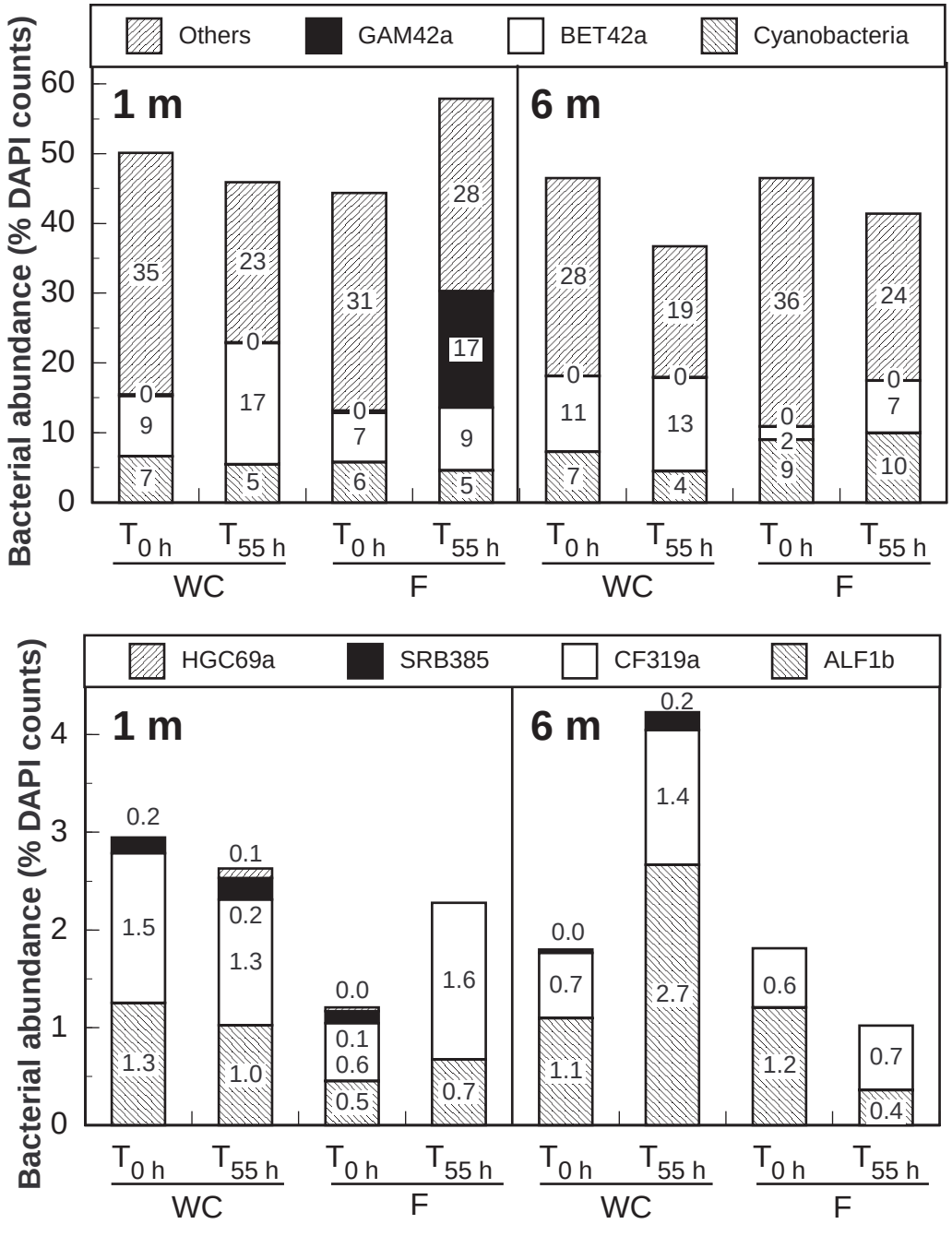

Fig. 4. Changes in the bacterial community composition during the incubations. $T_{0\ h}$, initial composition; $T_{55\ h}$, composition after 55 h; WC, whole community; F, fraction filtered on 1.2 μm membranes.

respiration rate of two lake communities over time; this effect was more pronounced in the more oligotrophic lake (Carignan *et al.*, 1998).

The changes in respiration in Lake Cadagno were not consistently correlated to changes in bacterial abundance or cell volume. Bacterial cell numbers significantly increased after 12–24 h of incubation and sharply declined afterwards. Similar changes have been reported in marine pelagic marine communities (Fuhrman and Azam, 1980; Pomeroy *et al.*, 1994). The decrease in bacterial abundance during the course of incubations could not result from the grazing activity because it was similar whether the population of grazers was intact (whole community samples) or selectively removed (filtered fraction).

The respiration rate increased or decreased, depending on the sample considered, despite a consistent increase in bacterial cell volume. Both Williams and Pomeroy *et al.* also reported that the respiration rate does not reliably reflect changes in the bacterial abundance and size (Williams, 1981; Pomeroy *et al.*, 1994).

It is probably more appropriate to relate changes in the respiration rate to changes in biomass. Estimation of changes in bacterial biomass is possible assuming that the carbon content per cell is proportional to cell volume. Such calculation suggests that the bacterial biomass increased considerably during incubations (average: 56%; range: 49–63%), except for the unfiltered samples collected at 6 m. In these samples, the increase in cell volume almost exactly compensated the decline in bacterial abundance; hence, the bacterial biomass did not change significantly. Note that we do not report absolute biomass values because the carbon content per cell is subject to considerable uncertainty (Ducklow, 2000). Estimates of percentage changes do not depend on its magnitude, assuming that it does not change during the course of the incubations. The bacterial biomass is, like bacterial abundance and bacterial cell volume, a poor predictor of respiration rates. For example, despite an increase in bacterial biomass greater than 50%, the respiration rate of the whole community decreased between the beginning and end of the incubations at 1 m whereas it increased at 6 m.

The bacterial counts and cell size were similar in filtered and unfiltered samples, indicating that the grazing activity of ciliates or zooplankton, which were selectively removed by filtration, did not play a major role in controlling the bacterial abundance and cell volume. A relatively large portion of the flagellates may also have been removed. This may explain the lack of a shift towards filamentous bacteria in the unfiltered samples during the incubation.

Previous experiments showed that there are very few changes in the composition of the microbial community in lake Cadagno on time-scales similar to those considered in the present paper (Tonolla, unpublished results). Therefore any alteration of the communities initially enclosed in the incubation bottles essentially results from a response to containment. Between 32 and 55% (average 42%) of the bacteria stained with DAPI hybridized with the eubacterial probe EUB338. The signal after *in situ* hybridization with fluorescently labeled probes is related to the ribosome content of the cell and hence to their activity (Amann *et al.*, 1990b). It is likely that the activity of planktonic bacteria is maintained at low levels due to the oligo-mesotrophic conditions of the upper mixolimnion of Lake Cadagno (Del Don *et al.*, 2001). However, the values reported for EUB338 in the present study compare very well with data previously reported by Glöckner *et al.* in several oligo- and mesotrophic lakes [29–64%; (Glöckner *et al.*, 1996)]. The β subclass of Proteobacteria is the dominant bacteria in surface waters of lake Cadagno. This group, which is virtually absent in marine ecosystems, dominates the bacterial community in most freshwater ecosystems [median 16%; (Glöckner *et al.*, 1999)]. The Archaea and sulphate-reducing bacteria were only observed in aggregates of organic matter in which anoxic microniches presumably occurred. The percentage of bacteria detected by the eubacterial probe exceeded the sum of bacteria detected by probes ALF1b, BET42a, GAM42a, CF319a, HGC69a and SRB385 (Figure 4), This result is in agreement with previous studies [e.g. (Alfreider *et al.*, 1996; Glöckner *et al.*, 1999)] and further stresses the need for new probes targeting presently undetectable Eubacteria in aquatic environments. The probes used enabled a better detection of bacteria at 6 m than at 1 m, perhaps because they were more active due to a greater availability of particulate and dissolved organic carbon at that depth (Bertoni *et al.*, 1998).

Bacterial community structure has been shown to be altered by bacterivory (Simek *et al.*, 1999; Suzuki, 1999), to change during the aging of laboratory-made aggregates (Grossart and Ploug, 2000) and during long-term (8–10 days) incubations (Massana *et al.*, 2001). Our results demonstrate that it also changes during shorter (< 55 h) incubations. The most prominent change is the consistently increased abundance of β-Proteobacteria, the dominant bacterial group, during the course of the experiments. Changes in community composition may result from the modification of substrate quality and quantity over time.

These results suggest that, in addition to changes in bacterial abundance and size, qualitative changes of the bacterial community occur during measurement of respiration of planktonic communities. Such shifts in the taxonomic assemblage were hypothesized to be one of the reasons explaining the lack of correlation between respiratory activity and quantitative descriptors of the marine bacterial community (Pomeroy *et al.*, 1994). Our results confirm that these shifts also occur in a freshwater system but the data are insufficient to demonstrate a causal link with changes in respiration or to explain its poor correlation with changes in bacterial abundance, cell volume, or biomass as reported in the present study and others (Williams, 1981; Pomeroy *et al.*, 1994). As pointed out by del Giorgio and Cole, the increased use of molecular techniques will soon enable us to open the black box of planktonic bacteria and determine the effect of changes in the taxonomic composition of the bacterioplankton assemblage on bacterial biogeochemical processes (del Giorgio and Cole, 2000). Several recent papers have

begun to document such effects (Ouverney and Fuhrman, 1999; Cottrell and Kirchman, 2000; Riemann *et al.*, 2000).

Discrete incubations are used to estimate the rates of respiration and net primary production of planktonic communities as well as to determine bacterial degradation of dissolved organic carbon, microbial respiration, bacterial growth efficiency, conversion factors of leucine and thymidine uptake, and various other bioassays. Sheer *et al.* have shown that incubated oceanic bacteria can attain rates of protein and DNA synthesis one order of magnitude greater than *in situ* (Sherr *et al.* 1999). The increase occurred after fairly long lag times (19 to 43 h). All parameters of microbial activity are potentially affected by alterations in the taxonomic make-up of the community. There is therefore a critical need to develop alternative techniques with no incubation or incubation. Such techniques are now available for investigating bacterial activity, for example the combined microautoradiography-16S rRNA probe technique for determination of radioisotope uptake by specific microbial cell types *in situ* (Ouverney and Fuhrman, 1999) and the MICRO-FISH approach (Cottrell and Kirchman, 2000). Similar developments are required for measuring respiration with a technique more sensitive than the oxygen technique in order to decrease the incubation time required to detect fluxes. That would avoid, as much as possible, having a bacterial community composition at the end of the incubation too different from the original one. The use of membrane inlet ion trap mass spectrometry (Cowie and Lloyd, 1999) to estimate rates of respiration may have the potential to achieve this goal.

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